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divisional application to the application mentioned
under INID code 62.

(54) **Proces for the preparation of a controlled release system**

(57) The present invention relates to a process for
the preparation of a system having good controlled re-
lease behaviour. It comprises two water-soluble poly-
mers and a proteinaceous material.

An aqueous two-phase system is formed, wherein
the proteinaceous material is comprised in the dis-
persed phase. The dispersed phase is cross linked.

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[0001] This is a divisional application of EP-A-0 941 068 the contents thereof being incorporated by reference in the present application.

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described a technique wherein loading levels higher than obtainable by solution sorption, hence higher than about 0.1 wt.%, can be achieved in purified, pre-formed hydrogels. The loading technique is based on the fact that certain polymer mixtures split into separate phases when dissolved in water. Proteins dissolved in such a system distribute unevenly between the phases. This principle also holds when one of the polymer phases is a crosslinked gel.

[0017] In particular, Gehrke et al. describe a crosslinked dextran gel/poly(ethylene glycol) system, and a crosslinked hydroxypropylcellulose gel/poly(vinyl alcohol) system. Proteins present in an aqueous solution containing beads of the gel are, after the addition of the non-crosslinked second polymer, adsorbed on the beads and partly absorbed through meshes or pores in the bead surfaces.

[0018] A disadvantage of this technique is that the proteinaceous material is to a major extent only adsorbed to the beads, which means that if the phase containing the second polymer is replaced by another aqueous system a fast removal of the proteins from the beads is observed. Only when large amounts of pores having a diameter larger than the size of the proteinaceous material to be loaded are present in the bead surfaces, some absorption may occur. This adsorption and limited absorption behaviour has an undesirable effect on the release of the proteinaceous material from the beads.

[0019] To additionally illustrate the undesired release behaviour, it is noted that the profiles shown in the article are - from a pharmacological point of view - entirely unsuitable to be used in controlled release systems. Moreover, the gel beads are too large (cylinders with a diameter of 1.5 mm) to be suitably used for administration in the human or animal body.

[0020] In EP-A-0 213 303 a method for producing spherical polymer particles from systems containing two liquid aqueous phases is described. One of the two phases is dispersed in the form of droplets in the other phase to form an emulsion. Subsequently, the droplets are caused to solidify. In the phase to be dispersed, a macromolecular substance may be dissolved. Further, low molecular substances such as medicaments, vaccines and insecticides can be chemically bonded to the particle forming substance in the dispersed phase. Nothing is being said about the release behaviour of the dissolved substance, nor over the application of the spherical polymer particles formed or the size thereof.

[0021] The principle of affinity partitioning in PEG-containing two-phase systems is also known from Göte Johansson, Affinity Partitioning in PEG-containing Two-phase Systems In: Topics in Applied Chemistry; Poly(ethylene glycol) chemistry, Biotechnological and Biomedical Applications, Ed. J.M. Harris, Plenum Press (1992). In this article, a two-phase system is described, which is created when an aqueous solution of dextran and polyethylene glycol (PEG) are mixed. A PEG enriched and a dextran enriched phase are formed. Proteins are partitioned unequally in such systems. These known systems are used in the purification of proteins.

[0022] The present invention is aimed at providing a new injectable, patient friendly delivery system for protein drugs, which system is safe and biodegradable, and which system possesses well controllable delivery kinetics. The period wherein drug delivery should be guaranteed depends on the protein drug used, and varies between a number of days upto more than one year. In addition, high degrees of loading in the delivery system should be obtained. Moreover, the system of the present invention should be produced without needing the use of organic solvents.

[0023] The problems mentioned above are solved by a specific preparation method of controlled release systems, such as microspheres, wherein water is used as the solvent. The use of water as sole solvent system is advantageous from an environmental point of view, because of toxicological considerations and, especially, because of reasons of protein stability.

[0024] In a first aspect, the present invention relates to a process for the preparation of a controlled release system, comprising:

(a) forming of an aqueous two-phase system from two water soluble polymers and at least one releasable compound, the two water soluble polymers being incompatible in solution, at least one of these polymers being crosslinkable, the crosslinkable polymer phase being emulsified in the other polymer phase; and the at least one releasable compound being soluble in the crosslinkable polymer phase in the aqueous solution;

(b) allowing the releasable compound to dissolve or diffuse in the crosslinkable polymer phase; and

(c) crosslinking of the crosslinkable polymer, wherein step (b) can be carried out before or after step (c).

[0025] In a preferred embodiment, the crosslinking is carried out to such a degree that the pores (meshes) in the crosslinked structure eventually formed are substantially smaller than the size of the releasable compound. By emulsifying an aqueous crosslinkable polymer in a continuous phase comprised of water and a polymer which is not compatible with the crosslinkable polymer, and crosslinking the discontinuous phase, the particle size of the crosslinked polymer particles can be adjusted, while the particle size distribution can be narrow, as is described herein-below in more detail.

[0026] Hence, in a further aspect, the present invention is directed to microspheres, at least 80 wt.% thereof having a particle size of between 100 nanometer and 100 µm, which microspheres are comprised of a degradable, crosslinked

polymer encapsulating at least one releasable compound, the pore size of the crosslinked polymer being equal or preferably smaller than the particle size of the releasable compound. These microspheres are obtainable by using the process of the invention, and are free from organic solvents. Dependent on the application of the microspheres, the size can e.g. be adjusted between 1 and 50 μm , preferably between 2 μm and 25 μm , such as between 5 and 15 μm .

[0027] When the pore sizes or meshes of the crosslinked polymer are equal or smaller than the hydrodynamic diameter size of the releasable component, the releasable component is essentially released when the polymer is degraded. More in particular, in this embodiment, the crosslinked structure must be degradable in the human or animal body, so that the encapsulated releasable compound can leave the crosslinked matrix. If on the other hand, the pore sizes or meshes of the crosslinked polymer are larger than the size of the releasable component, the releasable component is at least partially released by diffusion. The pore size of the crosslinked product obtained by the process of the present invention in this way provides a perfect tool to control the release. Further, the efficiency of the incorporation of a releasable compound in such a polymer structure is very high, while the degree of loading can be adjusted up to the saturation concentration of the compound to be released.

[0028] The degradability of the crosslinked structure can be regulated in a number of ways. As a first example, it is noted that bonds can be incorporated, which are hydrolysable under physiological conditions. In this respect, reference can be made to the European patent application 96201821.4 of the group of the present inventors. This patent application teaches hydrogels comprising hydrolytically labile spacers between different polymer chains. The hydrolytically labile spacers described therein can be suitably used in the present invention and are incorporated herein by reference.

[0029] Another example to control the degradability is the coencapsulation of an enzyme or chemical substance capable of breaking bonds in the crosslinked polymer. In a preferred embodiment of the process of the present invention the crosslinkable polymer is a dextran polymer. In this embodiment a dextranase can be added to the aqueous two-phase system before the crosslinking step or added afterwards.

[0030] The dextran polymer can suitably be used in the process of the present invention together with a polyethylene glycol or Pluronic®, which is a preferred polymer to be used in the inventive process.

[0031] The product aimed at by the process of the present invention can be separated from the other polymer phase using conventional techniques, for instance centrifugation and decantation.

[0032] In a first step of the process of the present invention an aqueous two-phase system is formed. This two-phase system comprises water, and at least two water soluble polymers, which polymers are incompatible in solution. Preferably a compound to be released is also present, although it is possible to add the compound to be released after the crosslinking step. At least one of the polymers present in the aqueous phase is crosslinkable, and the crosslinkable polymer phase is emulsified in the other aqueous polymer phase.

[0033] The polymers used can be chosen dependent on the nature of the compound to be released. It is preferred that the compound to be released will have a clear preference for the crosslinkable polymer phase. In that case, the highest possible degree of loading, theoretically up to the saturation concentration, in the microspheres to be made, can be obtained.

[0034] It is not critical which crosslinkable polymer is used. However, if the controlled release system comprising the polymer in crosslinked form is intended to be brought into a human or animal body, the polymer should be pharmaceutically acceptable and preferably should be degradable. Suitable crosslinkable water soluble polymers are dextrans and derivatized dextrans, starches and starch derivatives, cellulose derivatives such as hydroxyethyl and hydroxypropyl cellulose, polyvinylpyrrolidone, proteins and derivatized proteins, and so on. The molecular weight of the crosslinkable polymers used normally lies between 1,000 and 1,000,000 Da. It is noted that with a higher molecular weight of the polymer, a better phase separation is generally obtained in the aqueous solution used in the process of the invention.

[0035] The person skilled in the art will have the knowledge to choose the crosslinkable polymer and the crosslinking conditions required for the emulsion prepared. For instance, dextrans can be crosslinked with methylacrylate or methacrylate groups. Another example is a system comprising PVP as the external phase and dextran as the emulsified phase, wherein the dextran is crosslinked through the presence of isocyanates.

[0036] Further, reference is made to crosslinking using radiation. Dex-GMA can e.g. be polymerized using small dosages of γ -radiation, such as less than 0.1 Mrad. An advantage of this embodiment is that in one step sterile micro-particles can be obtained. Further, crosslinking by UV radiation and physical crosslinking using e.g. hydrophobic tails coupled to a polymer are possible techniques.

[0037] In a preferred embodiment, the crosslinkable polymer is a temperature sensitive polymer such as poly-N-isopropylacrylamide, which polymer can, e.g., be present as a graft on another polymer such as a dextran. Hydrogels of these polymers show increasing swelling behaviour at degreasing temperatures. This makes it possible that releasable material can easily penetrate in the hydrogel after the crosslinking reaction. By subsequently raising the temperature, e.g. to a value of 37°C, the meshes in the hydrogel shrink, thereby capturing the releasable compound.

[0038] The polymer which is present in the aqueous continuous phase can be any polymer which is incompatible with the crosslinkable polymer. Although this polymer may also be crosslinkable, but of course not under the reaction conditions used for the crosslinking of the discontinuous polymer phase, this is not preferred. Examples of suitable

polymers incompatible with the polymer to be crosslinked are poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) (in combination with e.g. dextrans and dextran derivatives, starches and starch derivatives, PVP, and water soluble cellulose derivatives).

[0039] The release of the releasable compound depends on a number of variables, which can be used to tailor the delivery as desired. One of these variables is the size of the microspheres. The size can be adjusted by carefully modifying the process circumstances and formulation parameters in the emulsifying step. For instance, the water content, the presence of hydrophobic groups on any one of the polymers or mixtures of polymers used, the viscosity of the continuous and discontinuous phase, and the electrical charge on the at least two polymers used are examples of tools to adjust the size of the microspheres or microparticles to be produced. In addition, emulsifiers can be added. Suitable emulsifiers are copolymers, preferably block-copolymers, of units of the two incompatible polymers, e.g. a block-copolymer of PEG and dextran, used to create the two-phase system.

[0040] To further guarantee a controlled release, the crosslinked polymer should preferably be degradable.

[0041] As said herein-above, it is important that the two water soluble polymers are incompatible with one another, so that a two phase system is obtained after the two polymers have been added to each other in an aqueous solution. Whether or not a two phase system will be obtained depends not only on the nature of the two polymers involved, but also on the conditions under which they are added. Factors that are relevant in this regard are the molecular weight of the polymers, their concentrations in the aqueous solution, the temperature at which they are added to one another, and so forth. It is part of the standard skills of the artisan to determine a phase diagram for any combination of polymers that can be used, and thus to choose suitable conditions for obtaining a phase separation.

[0042] In the attached fig. 9 a phase diagram of a water/PEG/dextran ternary system is shown as example. When the starting-composition is below the binodal (---), a one phase system is present, whereas above the binodal, two coexisting phases are formed: one enriched in polymer 1 (composition x_1) and the other enriched in polymer 2 (composition x_2). x_1 and x_2 are connected via a tie-line (____). All systems prepared using starting-compositions on the same tie-line separate into phases of constant composition. For a given starting-composition, the volume ratio of the coexisting phases x_1/x_2 equals y_2/y_1 .

[0043] As indicated herein-above, the releasable compound can be a protein drug. However, it is also possible to encapsulate pharmacon containing nanoparticles or microparticles, e.g. liposomes and iscoms. The encapsulation of this type of particles has the advantage of preventing the occurrence of a too fast release of the encapsulated compound, or, said in other words, burst-effects can be avoided in a more secure way.

[0044] The partition of the compound to be released is primarily determined by the nature of the polymers present in the aqueous two-phase system. This partition can be influenced, e.g. by adding salt to the aqueous system, or by adjusting the pH.

[0045] If the releasable compounds, such as proteins, are present during the crosslinking step, care should be taken that the integrity of the releasable compounds is secured. It should for instance be avoided that proteinaceous material is oxidized by initiator systems etc. In this light, it is noted that adverse effects can be avoided or minimized by minimalizing the amount of initiator, reducing the polymerization time or adding suitable antioxidantia, such as α -tocopherol.

[0046] The separation of the crosslinked structures enclosing the releasable compound from the other phase can be carried out in any conventional way. Preferably, the separation is effected by filtration or centrifugation. The crosslinked structures can subsequently be washed with water and dried. The drying step determines that a pharmaceutically acceptable product can be obtained, having a maintenance term of more than 2 years. A very preferred drying method is spray-drying, although the drying can also be suitably carried out using lyophilization.

[0047] The process of the present invention will now be further illustrated by the preparation of dextran microspheres using the water-in-water emulsion technique, and by the following, non-limiting examples.

Example 1

[0048] Polyethylene glycols (PEG) with varying molecular weights were obtained from Merck-Schuchardt, Germany. Glycidyl methacrylate derivatized dextrans (dex-GMA) with varying DS (degree of substitution; the number of methacrylate groups per 100 glucopyranose residues) were synthesized by a coupling reaction of dextran T40 and glycidyl methacrylate in DMSO (dimethylsulfoxide) using DMAP (N,N-dimethylaminopyridine) as a catalyst, essentially as described by Van Dijk-Wolthuis et al. in *Macromolecules* **28**, (1995) 6317-6322.

[0049] Dex-PEG was synthesized as follows. mPEG (monomethoxypolyethylene glycol, M 5000 g/mmol, 5 g, corresponding with 1 mmol hydroxyl groups) and CDI (carbonyldiimidazole, 162 mg, 1 mmol) were dissolved in 100 ml of anhydrous tetrahydrofuran. The solution was stirred overnight at room temperature, followed by the evaporation of the solvent under reduced pressure. Next, the CI (carbonylimidazole) activated mPEG was added to a solution of dextran T40 (1.7 g) and DMAP (0.35 g) in 50 ml DMSO. This solution was stirred for one week at room temperature. After neutralization of DMAP with HCl, the solution was extensively dialyzed against water and subsequently freeze dried.

The product was characterized by gel permeation chromatography and NMR. The degree of substitution amounted to 4 Dex-lactate-HEMA (DS 3) was synthesized as described in the copending European patent application No. 96201821.4.

[0050] Polyethylene glycol (PEG, varying molecular weight) was dissolved in 0.22 M KCl to a concentration of 12-40% (w/w). Dex-GMA was dissolved in 0.22 M KCl to a concentration of 10-40% (w/w). Both solutions were flushed with nitrogen for 10 minutes. Next, 4.75 ml of the PEG solution and 0.25 ml of the dex-GMA solution were mixed and vortexed (Winn Vortex-Genie, maximum speed) for 1 minute resulting in a water-in-water emulsion with dextran as the inner phase and PEG as the outer phase. After 10 minutes TEMED ((N,N,N',N'-tetramethylethylenediamine, 100 μ l, 20% (v/v) in 0.22 M KCl, pH adjusted with concentrated HCl to 7.2) and KPS (potassium peroxydisulfate, 180 μ l, 50 mg/ml in water) were added. The emulsion was incubated for 30 minutes at 37°C to polymerize the dex-GMA. The microspheres were washed twice with water and freeze dried.

[0051] It was demonstrated using *in vitro* cell cultures that cytotoxicity of dex-GMA is low in similar to the cytotoxicity of dextran, which compound has been used for years as plasma replacing agent in human beings.

[0052] The particle size (number weight diameter ($=\Sigma nd/\Sigma n$) and volume weight diameter ($=\Sigma nd^4/\Sigma nd^3$)), I.C. Edmundson, Particle-size analysis, H.S. Bean, A.H. Beckett and J.E. Cartes (eds) in: Advances in Pharmaceutical Sciences vol.2, Academic Press, London 1967, 95-174) and particle size distribution were determined by a laser light blocking technique (Accusizertm, model 770, Particle Sizing Systems, Santa Barbara, CA, USA). The shape and surface characteristics (porosity) of the microspheres were established by scanning electron microscopy (SEM) analysis.

[0053] Figure 1 gives a representative example of particle size distribution of a dextran microsphere batch prepared via the water-in-water emulsion technique as determined using the Accusizer. SEM analysis showed that the particles are perfectly spherical and non-porous (Figure 2).

[0054] Figure 3 shows the volume weight average diameter of dextran microspheres as a function of the degree of GMA substitution and the molecular weight of PEG. The concentration of the PEG solution was 24% (w/w); the concentration of the dex-GMA concentration was 20%. It is shown that the particle size increases with decreasing molecular weight of PEG. At a fixed molecular weight of PEG, the particle size slightly decreases with increasing DS.

[0055] Figure 4 shows the volume weight average diameter of dextran microspheres as a function of the degree of GMA substitution and concentration of the aqueous dex-GMA concentration. The mean diameter decreases with decreasing dex-GMA concentration.

[0056] Figure 5 shows the effect of the concentration and molecular weight of PEG on the volume weight average diameter of the dextran microspheres. For this evaluation, dex-GMA with a DS of 8 in 0.22 M KCl (20%, w/w) was used. It appears that for a given PEG, the largest particles were obtained at a PEG concentration around 24%.

Example 2

[0057] The water-in-water emulsion can also be prepared using a mechanical stirrer instead of the vortex used in Example 1. A solution of dex-GMA (DS 4,7,13 or 30; 20% w/w) in 0.22 M KCl was added to a solution of PEG (varying molecular weight; 24%) in 0.22 M KCl, and mechanically stirred (around 600-1000 rpm, depending on the viscosity of the PEG phase) for 5 minutes under a nitrogen stream. Next, TEMED ((N,N,N',N'-tetramethylethylenediamine, 1.0 ml, 20% (v/v) in 0.22 M KCl, pH adjusted with concentrated HCl to 7.2) and KPS (potassium peroxydisulfate, 1.8 ml, 50 mg/ml in water) were added and the mixture was incubated for 30 minutes at 37°C to polymerize the dex-GMA. The particle size is slightly greater using a mechanical stirrer instead of a vortex (Figure 5).

Example 3

[0058] Microspheres were prepared from the following formulations using the protocol as given in Example 1 and using the following stock solutions:

[0059] Stock solutions (% in w/w) in 0.22 M KCl:

- A. PEG 10.000, 24%
- B. PEG 20.000, 24%
- C. Dex-GMA (DS 13) 20%
- D. Dex-lactHEMA (DS 3) 20%
- E. Dex-lactHEMA (DS 3) 10%
- F. Dex-PEG 20%

Table 1

summarizes the results:				
PEG	dex	emulsifier	number weight diameter (μm)	volume weight diameter (μm)
4.50 ml A	0.25 ml C	0.25 ml F	3.3	7.2
4.75 ml A	0.25 ml C	no	4.4	11.5
4.75 ml B	0.25 ml D	no	6.3	17.0
4.75 ml B	0.25 ml E	no	5.3	16.0

[0060] As can be seen, a suitable emulsifier (block-copolymer of dextran and PEG) gives smaller particles with a smaller dispersity (= weight mean diameter/number mean diameter).

Example 4

[0061] The release of a model protein from non-degrading dextran microspheres and degradable microspheres was evaluated. The microspheres were rendered degradable by the incorporation of dextranase in dex-GMA microspheres.

[0062] Dex-GMA (DS 8) was dissolved in 10 mM phosphate buffer pH 8.0. To 2 ml of this solution a fixed amount of IgG (Immunoglobuline G, 25.6 mg) and a variable amount of dextranase (Sigma D1508; 0, 0.1 and 1 U (1 U releases 1 μmol reducing oligosaccharides per minute at 37°C and pH 6.0)) dextranase were added. This solution was emulsified in an aqueous solution of PEG (M 10.000, concentration 24% (w/w)) in 0.22 M KCl. Thereafter, TEMED (N,N,N',N'-tetramethylethylenediamine, 100 μl , 20% (v/v) in 0.22 M KCl, pH adjusted with concentrated HCl to 7.2) and KPS (potassium peroxydisulfate, 180 μl , 50 mg/ml in water) were added. The microspheres were washed with water and dried under a nitrogen flow.

[0063] An accurately weighed amount (0.3-0.5 g) of microspheres was suspended in 10 ml phosphate buffer pH 5.5 and the amount of protein released in the buffer was determined using the biorad protein assay (M. Bradford. Anal. Biochem. 72 (1976) 248-254). Figure 7 shows the release profiles. From this figure it is clear that the release of IgG from dextran microspheres can be modulated by dextranase.

Example 5

[0064] The release of a model protein (IgG) from degrading dextran microspheres was evaluated. Degradation was established by co-entrapment of dextranase in the microparticles.

[0065] Glycidyl methacrylate derivatized dextran (Ds=8: 138 mg) was dissolved in 612 μl buffer (10 mM phosphate, 220 mM KCl, pH 8.0). Thereafter, 250 μl of an aqueous solution of IgG (50 mg/ml) and 250 μl of an aqueous solution of dextranase (variable concentration) were added. IgG and Dextranase were dissolved in the same buffer (10 mM phosphate, 220 mM KCl, pH 8.0). Next, 500 μl of the dexMA. IgG. Dextranase solution was added to 5 ml of an aqueous solution of PEG (molecular weight 10.000 g/mol, concentration 17.5, 24 or 30% w/w) in the same buffer. These phase separated systems were vortexed for 1 minute, followed by the addition of 180 μl potassium peroxodisulphate (50 mg/ml; dissolved in phosphate buffer) and TEMED (N,N,N',N'-tetramethylethylenediamine, 20% v/v, pH adjusted to 8.0 with HCl). Next, the samples were incubated for 30 minutes at 37°C to polymerize the DexMA. The particles were collected by centrifugation and washed with water. The particles were resuspended in buffer (5 mM NH_4Ac , pH 5.5) and incubated at 37°C. Periodically, samples were withdrawn and analyzed for their protein content (Biorad assay). Figure 8 shows the release profiles. It can be seen that in the absence of dextranase the cumulative release was less than 10%, indicating that the hydrodynamic diameter of protein was larger than the hydrogel mesh size. Further, the release rate increases with increasing amount of dextranase in the particles. An increasing amount of dextranase in the particles resulted in an increasing degradation rate. This means that the release of entrapped proteins from dextran particles can be modulated by the degradation rate of the hydrogel matrix. Degradation can be established by addition of an enzyme (dextranase) or by the introduction of hydrolytically labile spacers (e.g. lactate esters) in the crosslinks.

Comparative Example

[0066] 5 grams of dextran in which the dextran chains are derivated with acryl groups, having a M_w of 40,000, was dissolved in 45 ml of water. A second solution was prepared, which solution comprised 7 g of polyethylene glycol having a M_w of 6,000 in 45 ml water.

[0067] At room temperature, the first solution was added to the second solution while stirring. The result was a one phase system, from which no microspheres could be formed. This example illustrates the need to choose the molecular

weights and concentrations of the starting materials in such a way that a two-phase system is obtained.

Claims

1. Process for the preparation of a controlled release system, comprising:

(a) providing:

- (aa) a first water-soluble polymer, said first polymer comprising crosslinkable groups, and incorporating bonds which are hydrolysable under physiological conditions;
- (bb) a second water-soluble polymer, being incompatible with the first polymer in an aqueous solution; and
- (cc) a releasable proteinaceous material;

(b) forming an aqueous two-phase system comprising a dispersed phase which comprises the first polymer, and a continuous phase which comprises the second polymer;

(c) allowing the releasable proteinaceous material to dissolve in or diffuse into the dispersed phase;

(d) crosslinking the first polymer to obtain microspheres, at least 80 wt.% thereof having a particle size of between 100 nanometer and 100 μ m, wherein step (c) can be carried out before or after step (d).

2. The process of claim 1, wherein the crosslinking is carried out to such a degree that the pores in the crosslinked structure are substantially smaller than the size of the releasable proteinaceous compound.

3. The process of claim 1 or claim 2, wherein before or after the crosslinking step an enzyme capable of degrading the crosslinked structure formed or to be formed is added to the aqueous two-phase system.

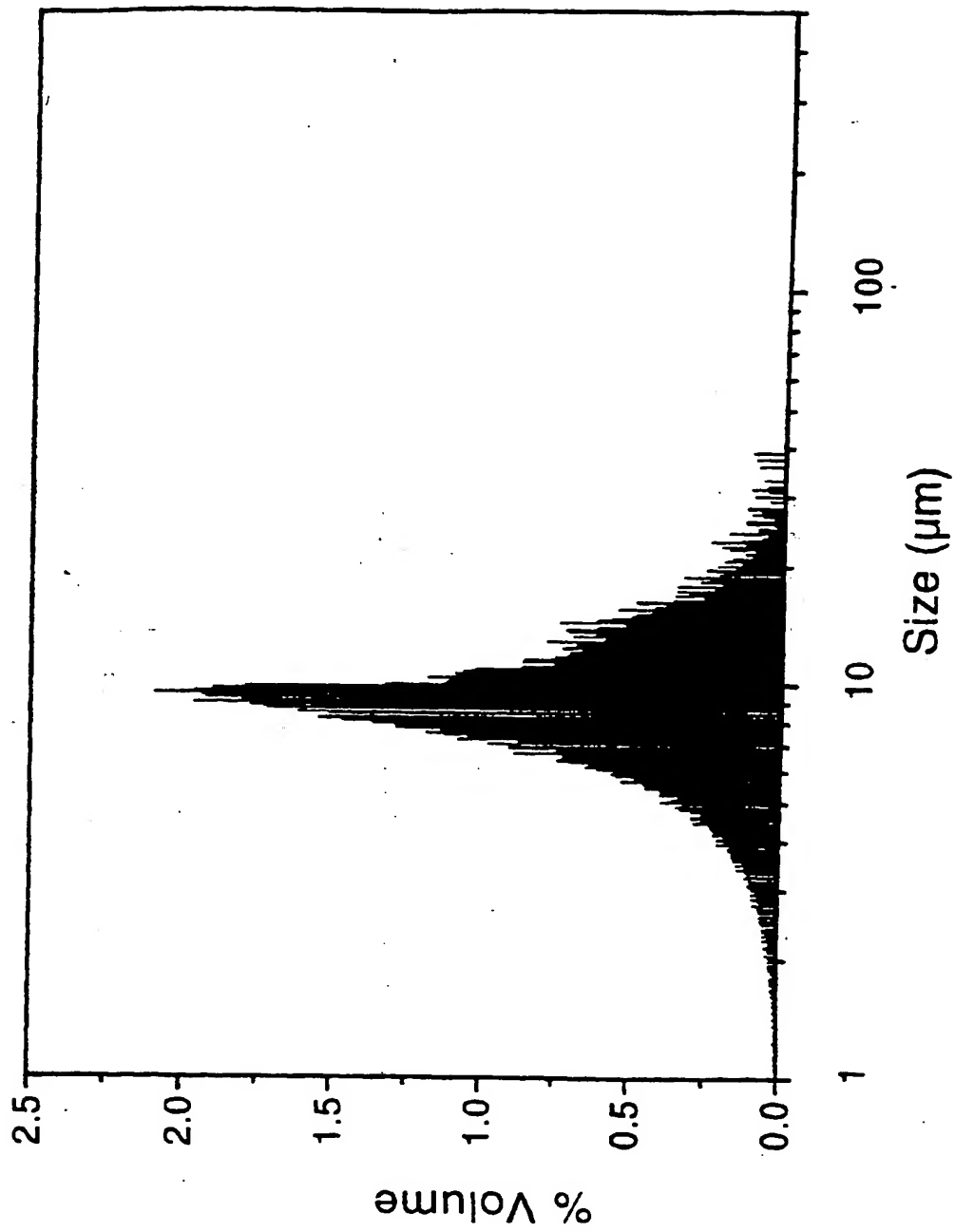
4. The process of any one of the preceding claims, wherein the crosslinkable polymer is a dextran polymer.

5. The process of claim 4, wherein before or after the crosslinking step dextranase is added to the aqueous two-phase system.

6. The process of any one of the preceding claims, wherein one of the water soluble polymers is polyethylene glycol.

7. The process of any one of the preceding claims, wherein the crosslinked structures are separated from the other polymer phase.

Figure 1
Volume particle size distribution
of dextran microspheres



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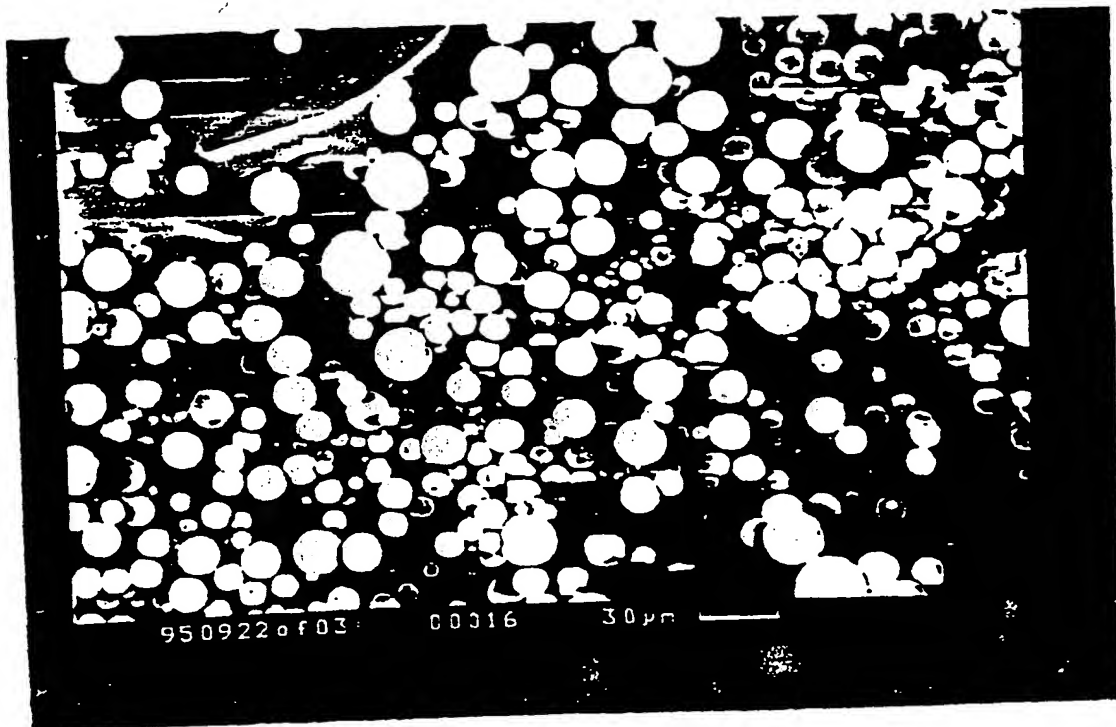


Figure 2

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Figure 3

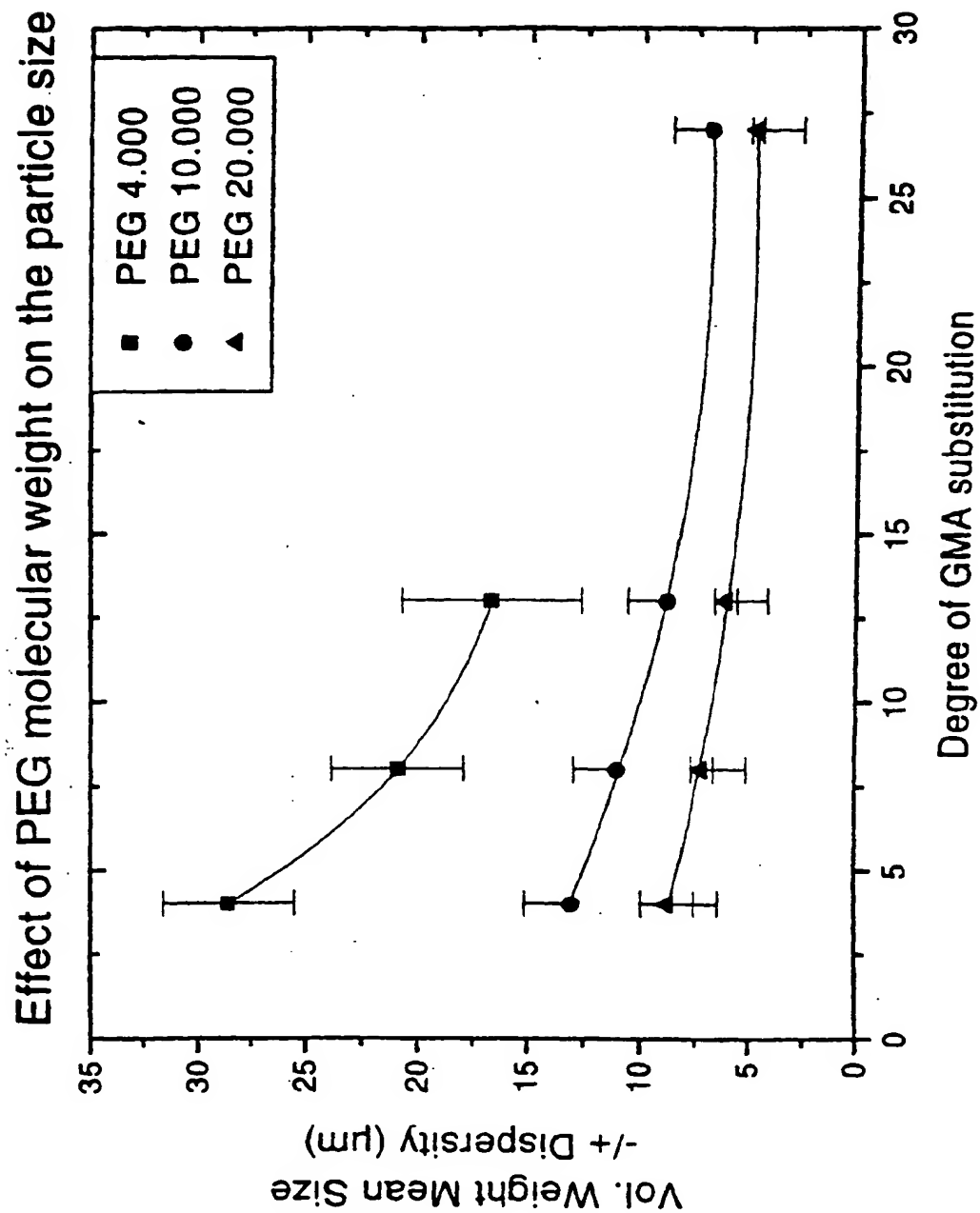


Figure 4

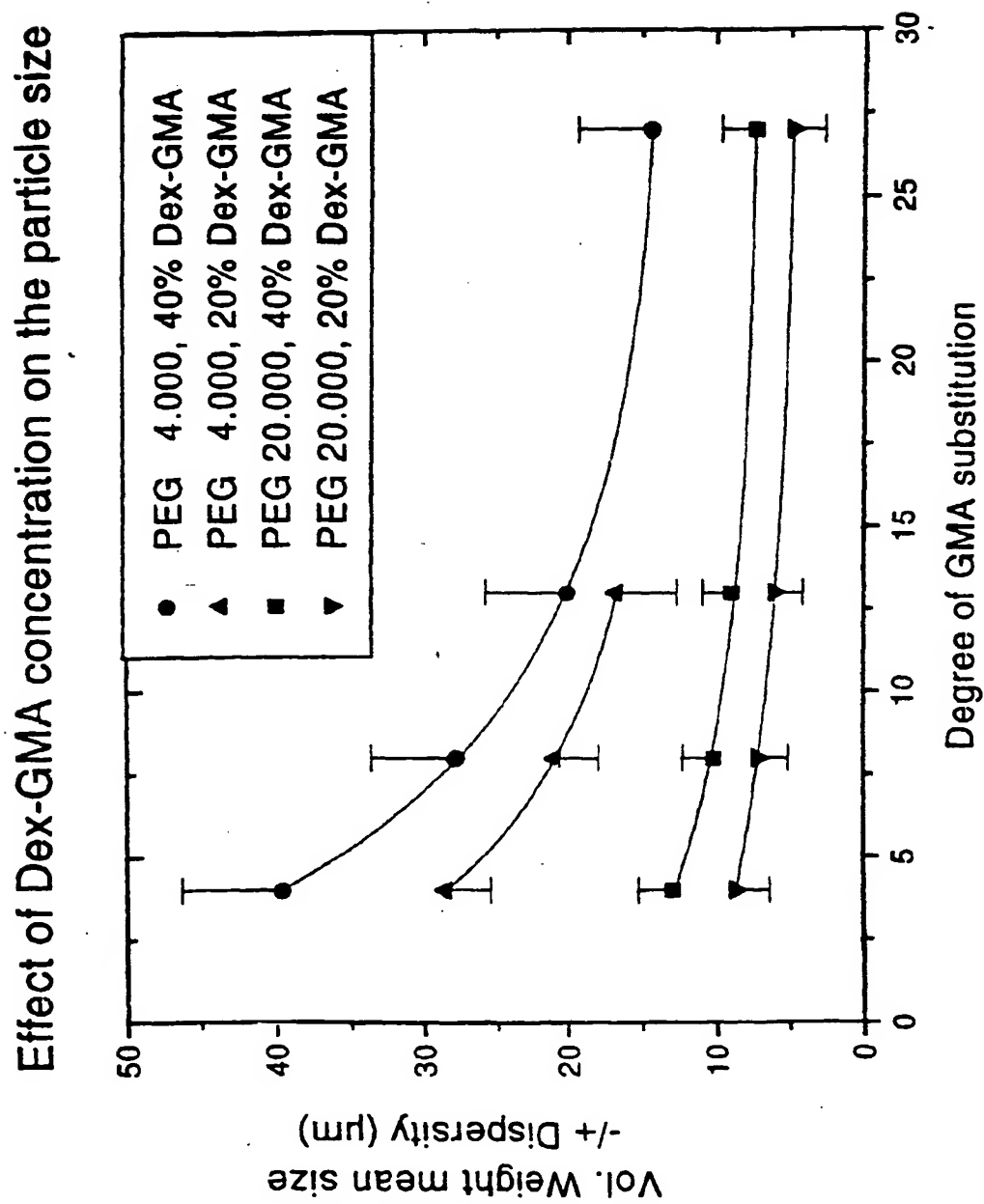
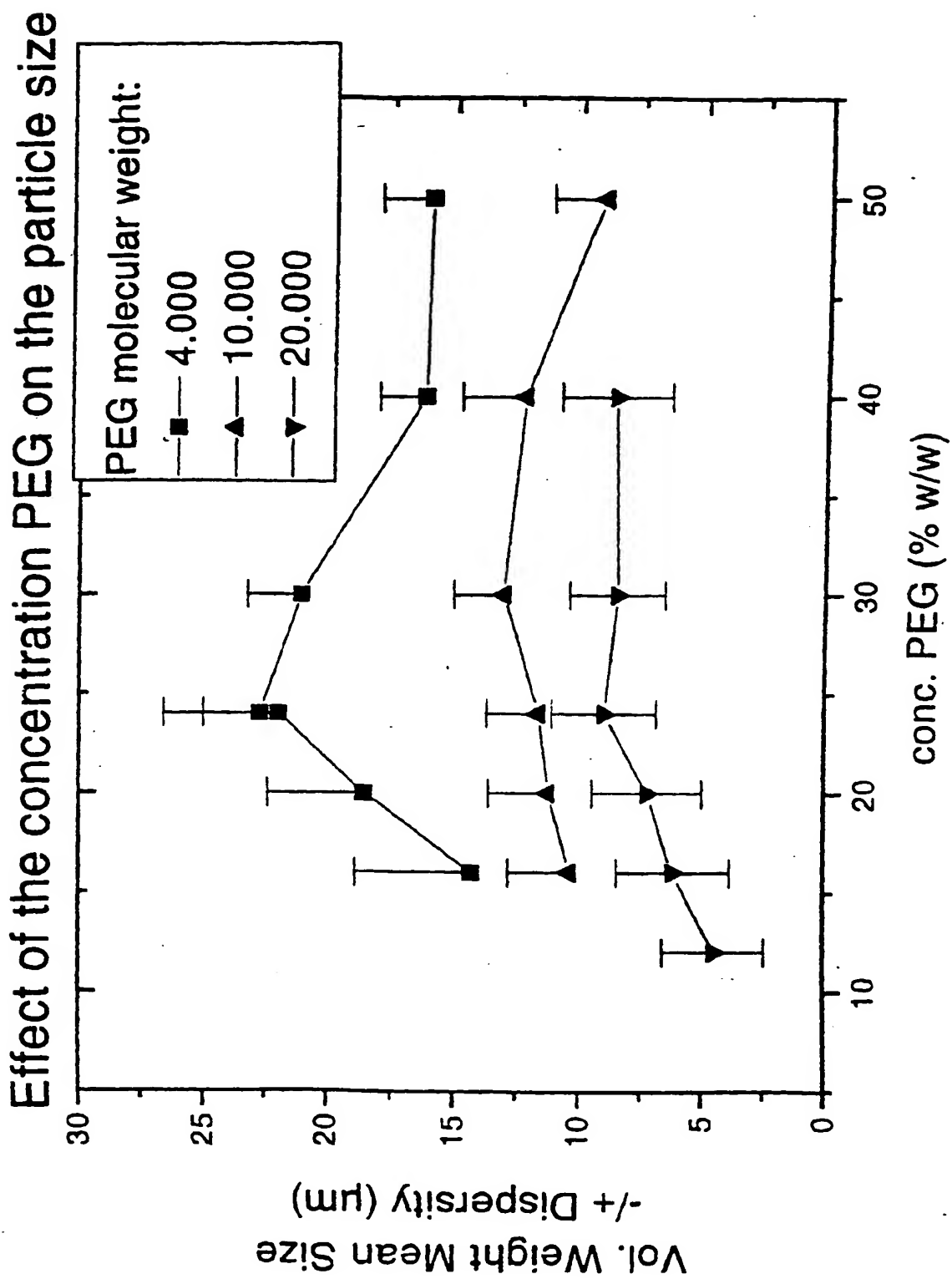


Figure 5



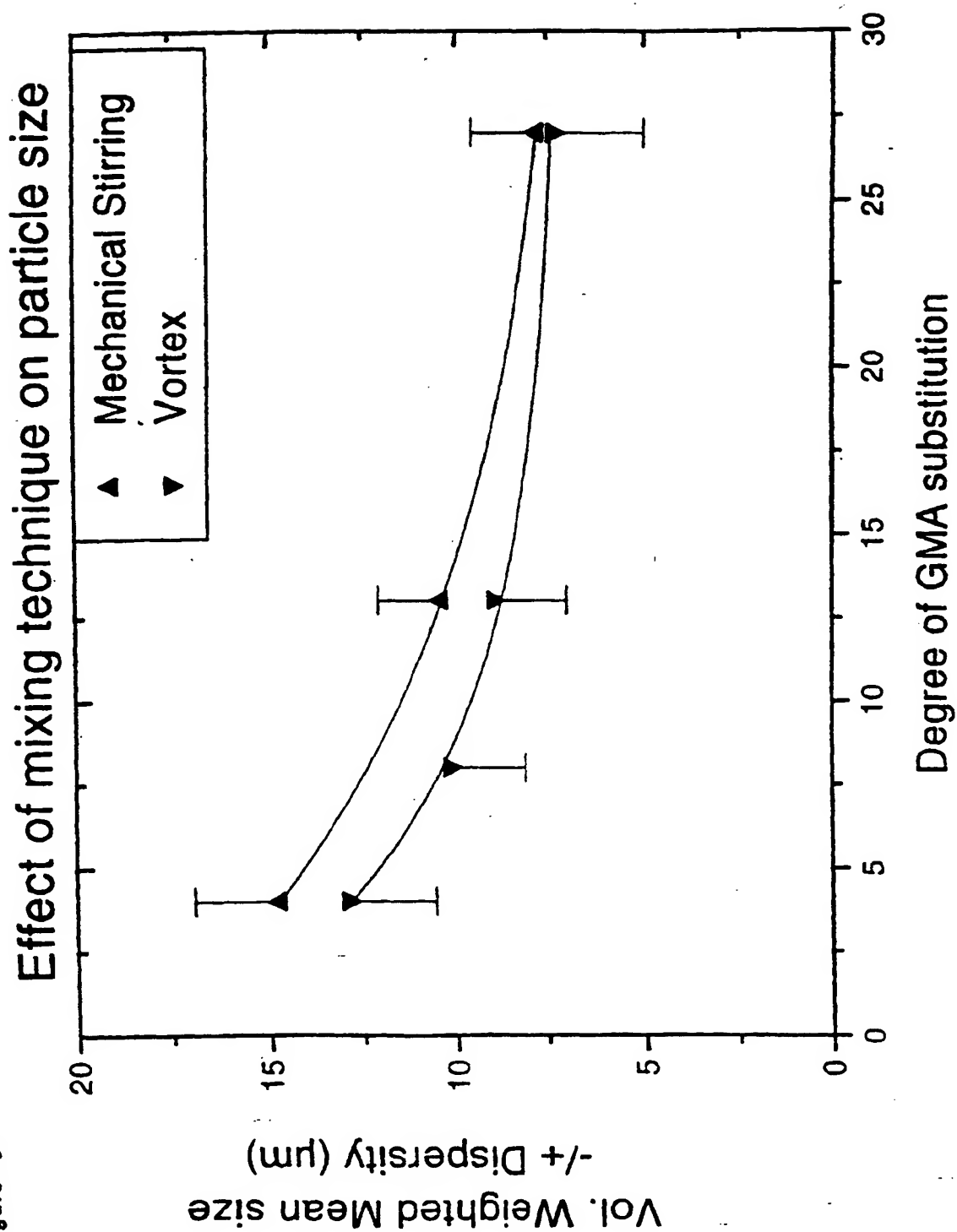
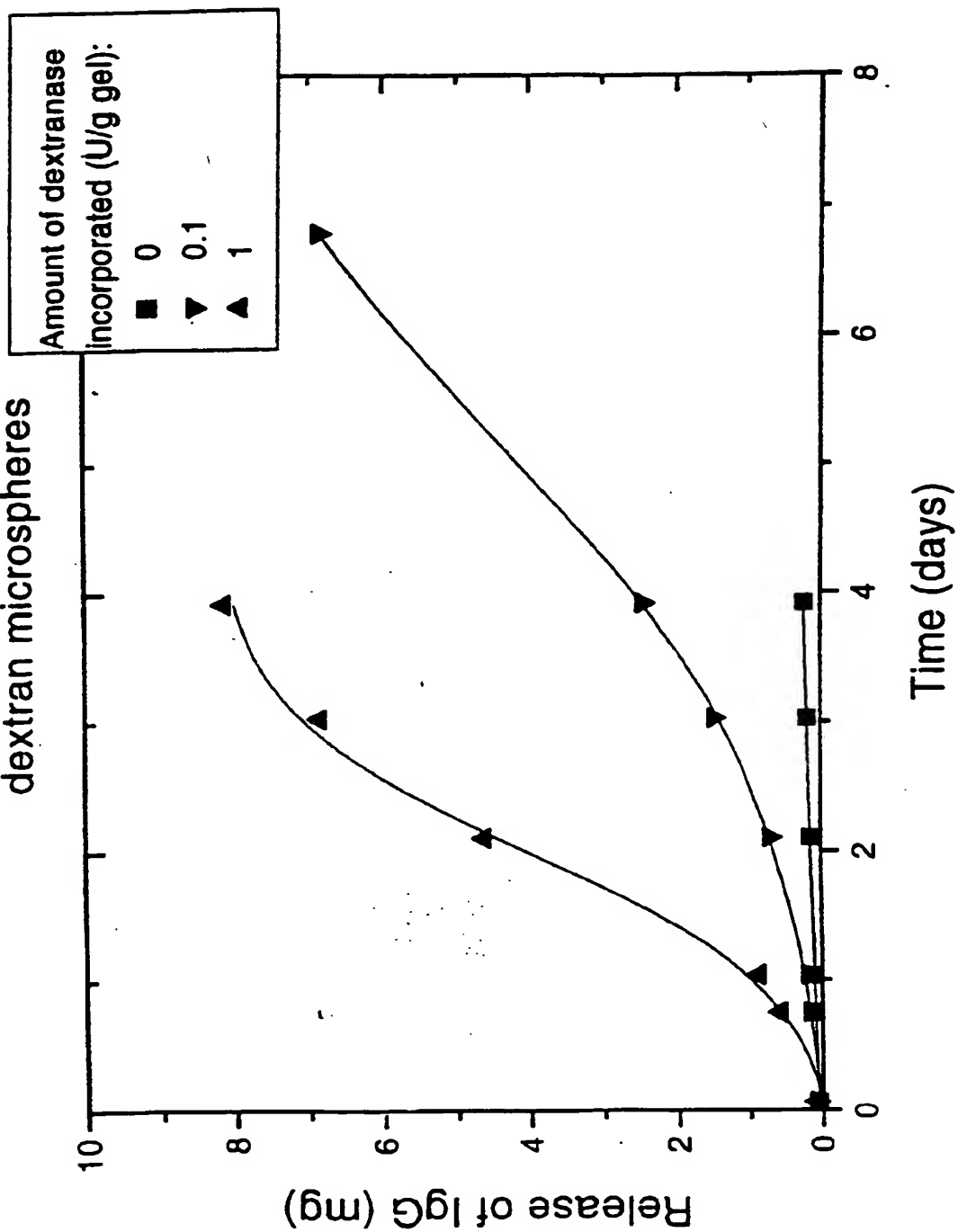
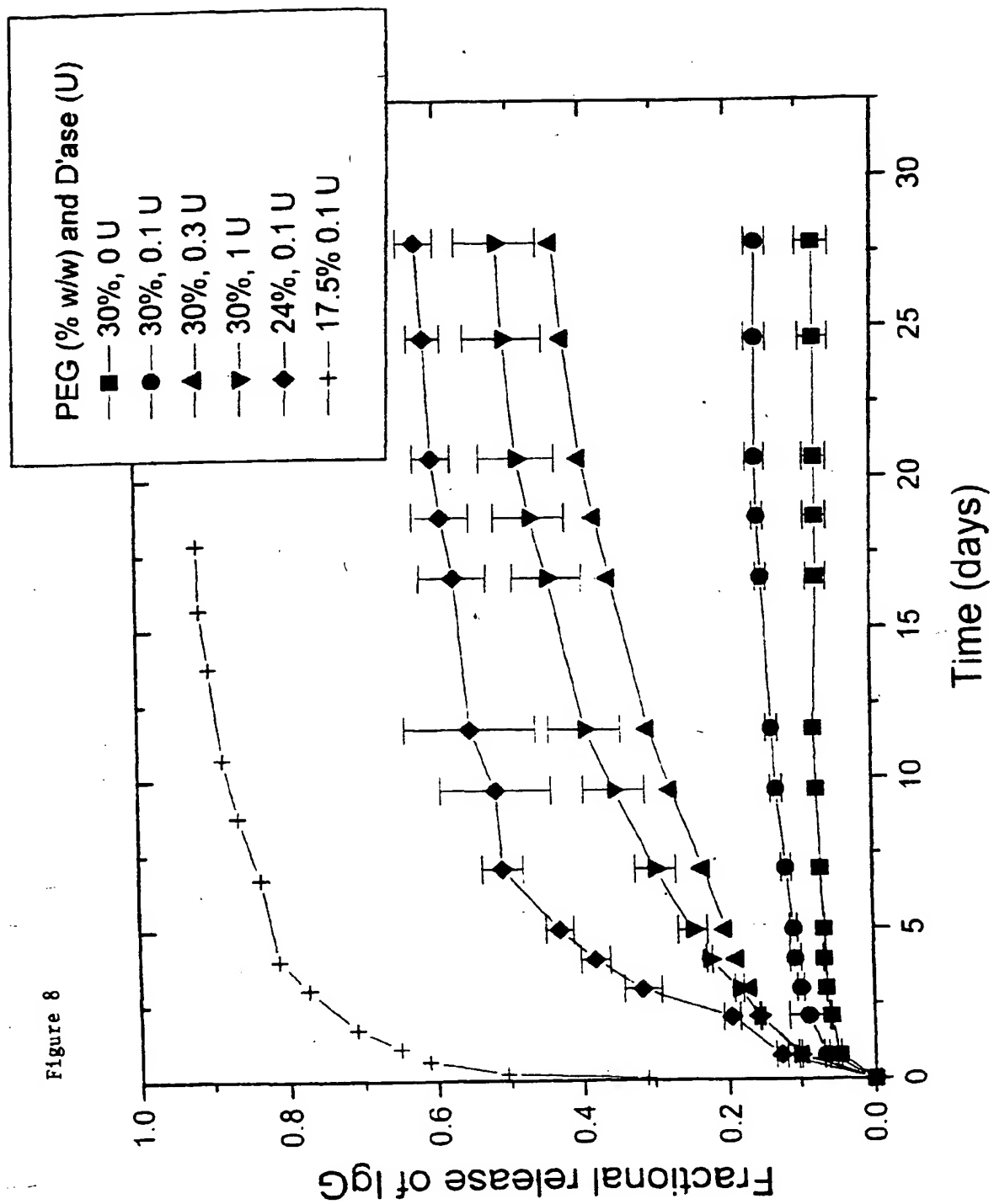


Figure 6

Release of IgG from enzymatically degrading
dextran microspheres



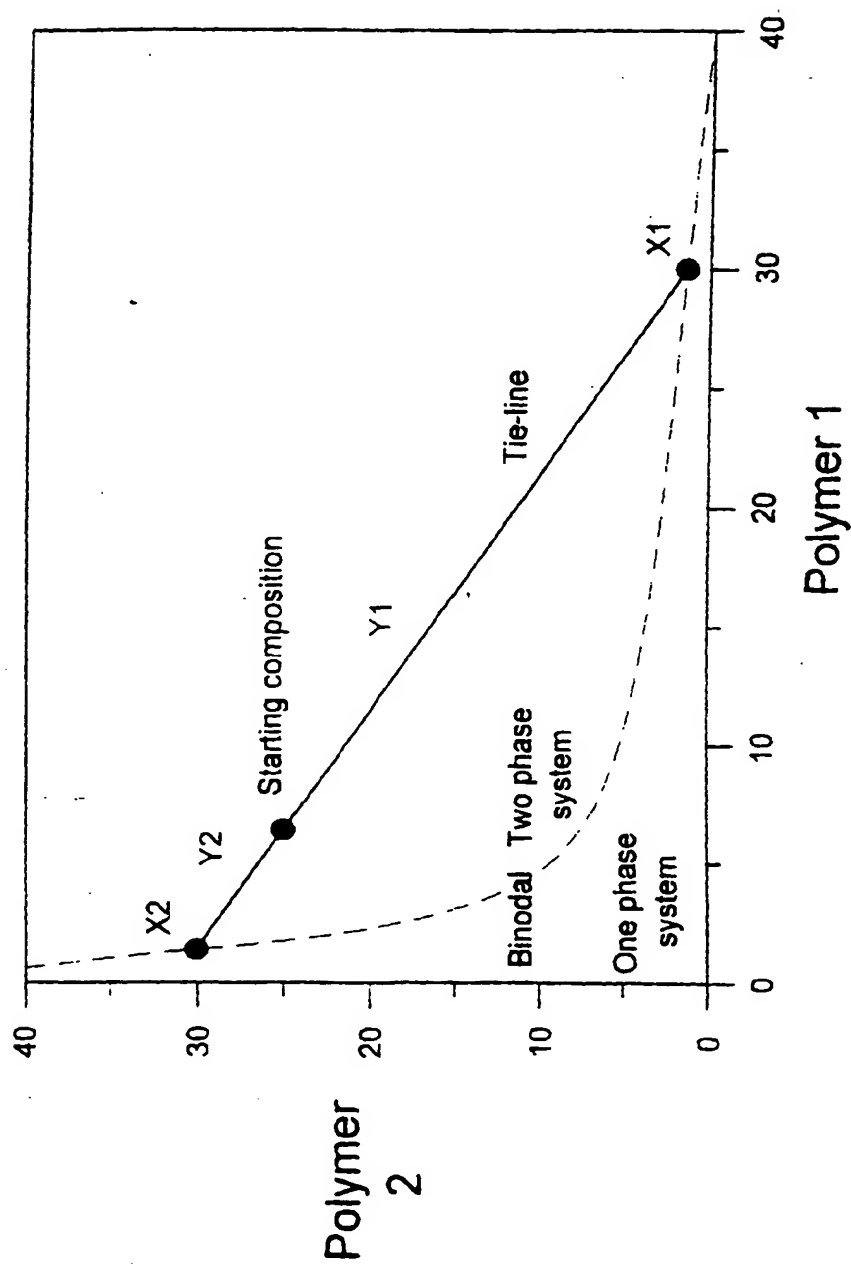


Fig. 9



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 07 7121

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X,D	EP 0 213 303 A (MAGNUS ET AL.) 11 March 1987 (1987-03-11)	1,2,6,7	A61K9/16
Y	* column 1, line 1 - line 7 * * column 7, line 21 - line 44 * * column 10, line 21 - line 28 * * column 13; example 6 * * claim 10 *	3-5	
Y	--- WO 95 34328 A (ROYER) 21 December 1995 (1995-12-21) * page 6, line 18 - line 27 * * page 14, line 1 - line 8 * * page 15, line 24 - line 25 * * page 22; example 6; table 3 *	3,5	
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